

REMARKS

Applicants have amended the Specification solely to correct a single typographical error and to identify the sequences disclosed in the Specification by their respective SEQ ID NOs as found in the Sequence Listing being submitted concurrently herewith. No new matter is introduced by virtue of these amendments, and the amendments are fully supported by the Specification of the subject application and the claims as originally filed. Accordingly, Applicants kindly request that the present Amendments be entered into the instant application.

Respectfully submitted,

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Enclosures



Exhibit A

Marked Up Versions of Amended Paragraphs
(Additions are double underlined, deletions are bracketed)

On page 18, please replace the paragraph beginning, "FIG. 10." with the following paragraph:

--FIG. 10. Diagrammatic representation of a PCR-directed mutagenesis method which can be used to replace viral coding sequences within viral gene segments. [(SEQ ID NOS: 52 and 58)]--

On page 18, please replace the paragraph beginning, "FIG. 11 (A)." with the following paragraph:

--FIG. 11.(A). Diagrammatic representation of relevant portions of pIVCAT1. The various domains are labeled and are, from left to right; a truncated T7 promoter; the 5' nontranslated end of influenza A/PR/8/34 virus segment 8 (22 nucleotides); 8 nucleotides of linker sequence; the entire CAT gene coding region (660 nucleotides) the entire 3' nontranslated end of influenza A/PR/8/34 virus segment 8 (26 nucleotides); and linker sequence containing the HgaI restriction enzyme site. Relevant restriction enzyme sites and start and stop sites for the CAT gene are indicated. (B) The 716 base RNA product obtained following HgaI digestion and transcription of pIVACAT1 by T7 RNA polymerase. Influenza viral sequences are indicated by bold letters, CAT gene sequences by plain letters, and linker sequences by italics. The triplets -- in antisense orientation --representing the initiation and termination codons of the CAT gene are indicated by arrow and underline, respectively (SEQ ID NOS: 52 and 58).--

On page 20, please replace the paragraph beginning, "FIG. 16." with the following paragraph:

--FIG. 16. Diagram of relevant portions of the neuraminidase (NA) gene contained in plasmids used for transfection experiments. The pUC19 derived plasmid pT3NAv contains the influenza A/WSN/33 virus NA gene and a truncated promoter specifically recognized by bacteriophage T3 RNA polymerase. The T3 promoter used is truncated such that the initial transcribed nucleotide (an adenine) corresponds to the

5' adenine of the WSN NA gene. At the 3' end of the cDNA copy of the NA gene, a Ksp632I restriction enzyme site was inserted such that the cleavage site occurs directly after the 3' end of the NA gene sequence (SEQ ID NO: 55). A 1409 nucleotide long transcript was obtained following Ksp632I digestion and transcription by T3 RNA polymerase of PT3NAv (as described in Section 8.1, infra). The 15 5' terminal nucleotides, the 52 nucleotides corresponding to the region between the restriction endonuclease sites NcoI and PstI and the 12 3' terminal nucleotides are shown (SEQ ID NOS:[54 and 61] 53, 59, and 55). The transcript of pT3NAv mut 1 (SEQ ID NO: 54) is identical to that of pT3NAv (SEQ ID NO: 53) except for a single deletion, eleven nucleotides downstream from the 5' end of the wild type RNA [(SEQ ID NO: 59)]. The transcript of the pT3NAv mut 2 is identical to that of pT3NAv except for 5 mutations located in the central region (indicated by underline)(SEQ ID NO: 61). These five mutations do not change the amino acid sequence in the open reading frame of the gene. The serine codon UCC at position 887-889 (plus sense RNA) was replaced with the serine codon AGU in the same frame. The numbering of nucleotides follows Hiti et al., 1982, J. Virol. 41:730-734 [(SEQ ID NO: 61)].--

On page 99, please replace the paragraph beginning, "Immunostaining of Infected cells" with the following paragraph:

--Immunostaining of Infected cells. Confluent MDCK monolayers in a 96-well plate were infected with transfectant or wild-type influenza viruses at an MOI > 2. Nine hours postinfection, cells were washed with PBS and fixed with 25 µl of 1 % paraformaldehyde in PBS. Then, cells were incubated with 100 µl of PBS containing 0.1 % BSA for 1 hour, washed with PBS three times, and incubated 1 h with 50 µl of PBS, 0.1 % BSA containing 2 µg/ml of the human monoclonal antibody 2F5. This antibody recognizes the amino acid sequence Glu-Leu-Asp-Lys-Trp-Ala (ELDKWA)(SEQ ID NO: 46) which is present in the ectodomain of gp41 of HIV-1 (Muster, T. et al., 1993, J. Virol. 67:6642-6647). After three PBS washings, 2F5-treated cells were incubated with 50 [µl] µl of PBS, 0.1 % BSA, containing a 1:100 dilution of a peroxidase-conjugated goat antibody directed against human

immunoglobulins (Boehringer Mannheim). Finally, cells were PBS-washed three times, and stained with a peroxidase substrate (AEC chromogen, Dako Corporation).--

On page 104, please replace the paragraph beginning, "Since the NA" with the following paragraph:

--Since the NA is required for viral infectivity in MDBK cells, transfectant viruses GP2/BIP-NA and HGP2/BIP-NA were able to express the NA protein in infected cells from their bicistronic NA genes. In order to study the expression of the GP2 and HGP2 polypeptides in transfectant virus-infected cells, infected MDCK cells were immunostained using the human monoclonal antibody 2F5. This antibody is specific for the amino acid sequence ELDKWA (SEQ ID NO:46) of gp41, which is present in the polypeptides GP2 and HGP2. The results are shown in Fig. 25. Wild-type WSN virus-infected cells did not stain with the gp41 -specific antibody 2F5. In contrast, both GP2/BIP-NA and HGP2/BIP-NA virus-infected cells showed positive staining with the 2F5 antibody. These results indicate that the first cistron (GP2 or HGP2) of the NA RNA segment of these two transfectant viruses is expressed in infected cells.--